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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : D21C 5/00

A1

(11) International Publication Number: WO 00/20677

(43) International Publication Date: 13 April 2000 (13.04.00)

(21) International Application Number: PCT/US99/23380

(22) International Filing Date: 6 October 1999 (06.10.99)

(30) Priority Data: 09/169,235 8 October 1998 (08.10.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**

*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: DELIGNIFIED SOYBEAN HULL FIBER AND METHODS OF MAKING AND USING

(57) Abstract

The present invention is a soybean hull fiber from which polyphenolics or lignin has been removed, having cellulose and hemicellulose, wherein the improvement is that the cellulose and hemicellulose are dissociated as a delignified soybean hull fiber. A method of delignifying the soybean hull fiber, has the steps of: (a) exposing the soybean hull fiber to a liquid having water and oxidizer in a non–basic solution thereby delignifying the soybean hull fiber into a delignified soybean hull fiber; and (b) separating the delignified soybean hull fiber from the liquid. The delignified soybean hull fiber is useful for adding to cellulose in papermaking and for generating other food, biochemical, and enzyme products. The method of the present invention may be extended to add an enzyme to the delignified soybean hull fiber for making cellulose derived compounds.

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# DELIGNIFIED SOYBEAN HULL FIBER AND METHODS OF MAKING AND USING

## FIELD OF THE INVENTION

The present invention is a soybean hull fiber that has had the lignins removed. In addition, the method of removing the lignin and the methods of using the delignified soybean hull fiber are part of the present invention.

## BACKGROUND OF THE INVENTION

Soybeans are used extensively in the food industry and are the basis for many food products including, for example tofu, non-dairy ice cream, and meat substitutes. In the process of shelling the soybeans the starch and oil is removed, and the soybean hull is a waste material that is disposed of or used as animal feed or burned.

Other hulls and fibers have been converted to useable fiber by removal of lignin.

For example, U.S. Pat. No. 5,705,216 to Tyson, is directed to a delignification process for delignification of woody and non-woody lignocellulosic biomass from agricultural waste products such as nut shells, seed hulls, and corn cobs. Tyson's process utilizes continuous extrusion reaction technology and high-pressure steam injection of an alkaline slurry to chemically and physically modify the lignocellulosic biomass but does not require the use of hydrogen peroxide. In Tyson, the term "non-woody" includes organic plant material comprising no more than about 20% lignin, while the term "woody" encompasses all other wood-like lignocellulose biomass materials. A disadvantage of this process is that it is operated at high pressure and temperature.

U.S. Pat. No. 5,023,097 to Tyson, is directed to a delignification process for delignification of non-woody (less than about 20% lignin) lignocellulosic

biomass from agricultural waste products. The process applies extrusion technology to hydrogen-peroxide-and-alkali-treated biomass in the presence of heat and pressure. Again, a disadvantage of this process is that it requires high pressure and temperature.

U.S. Pat. No. 5,656,129 to Good et al., is directed to a process of refining straw into fibers for use in board products. The process breaks down straw into individual fibers capable for use in dry, wet-dry, or wet processing to produce cellulosic materials such as fiberboards. The process involves the steps of cutting, softening, and refining straw by contacting it with steam under elevated pressure.

00143883, PAPERCHEM NO: AB5005812, Bioconversion Of Wheat-Straw And Wheat-Straw Components Into Single-Cell Protein, discusses increasing protein production from whole straw by removing the lignin component with a mixture of sodium chlorite and AcOH. A disadvantage to this process is that it requires a mixture of sodium chlorite and AcOH.

00269569, PAPERCHEM NO: AB6003388, Dissolving Pulps from Wheat Straw by Alkaline Hydrogen Peroxide Pulping, discusses dissolving pulps from wheat straw by alkaline pulping with various HOOH concentrations which vary pulp yield, degree of delignification, and degree of whiteness and bleachability. A disadvantage of this process is that it requires alkaline hydrogen peroxide treatments.

The article by Dusterhoft et al., Parameters Affecting The Enzymatic-Hydrolysis Of Oilseed Meals, Lignocellulosic By-Products Of The Food-Industry, Bioresource Technology, 1993, V44, N1, P39-46, discusses enzymatic hydrolysis of cell-wall materials (CWM) from sunflower and palm-kernel meals and partial delignification of the CWM using sodium chlorite and alkaline peroxide treatments. A disadvantage is that full delignification is not achieved with this process.

The article by Jung et al., Cell-Wall Composition And Degradability Of Forage Stems Following Chemical And Biological Delignification, Journal Of The Science Of Food And Agriculture, 1992, V58, N3, P347-355, discusses chemical and biological delignification methods used in treating stem material from

lucerne, smooth bromegrass and maize stalks with alkaline hydrogen peroxide, potassium permanganate, sodium chlorite, sodium hydroxide, nitrobenzene, and Phanerochaete chrysosporium. Again, a disadvantage of this process is that it does not achieve full delignification with sodium chlorite treatment.

The article by Dusterhoft et al., Nonstarch Polysaccharides From Sunflower (Helianthus-Annuus) Meal And Palm Kernel (Elaeis-Guineenis) Meal Preparation Of Cell-Wall Material And Extraction Of Polysaccharide Fractions, Journal Of The Science Of Food And Agriculture, 1991, V55, N3, P411-422, discusses two different chemical methods, sequential extraction with alkali and sodium chlorite, and treatment with 4-methylmorpholine N-oxide (MMNO). The methods were applied to the extraction of non-starch polysaccharides (NSP) from the enzymically deproteinated, water-insoluble cell wall materials of sunflower (Helianthus annuus L) meal and palm kernel (Elaeis guineensis Jacq) meal. A disadvantage with this process is that it requires a combined treatment using alkali and chlorite followed by treatment with MMNO.

The article, Effect of Biological Treatment on Cellulose Digestion, Papiripar 33, no. 5: 172-175 (1989), by Lepenye et al., discusses the effects of exposure to 30 microorganisms on the subsequent delignification of three LC substrates, viz., hemp, wheat straw, and poplar (Populus) chips, with sodium chlorite. A disadvantage of this process is that it requires exposure to microorganisms followed by sodium chlorite treatment, with the degree of delignification depending strongly on the individual substrates and organisms used.

The article, In Vitro And In Vivo Evaluations Of Soybean Residues Ensiled With Various Additives, J. Animal Sci. 49, no. 6: 1545-1551 (1979), Miller et al., discusses in vitro digestibilities of cellulose and organic matter using ensiled soybean residues (leaves, stems, and pods) treated with ammonium hydroxide, propionic acid, sodium chlorite, and wood molasses. The process has a maximum 70% moisture in the soybean residues and the separated lignin is not removed from the soybean residues thereby inhibiting enzymatic action on the soybean residues. A disadvantage of the process discussed in this paper is that

the authors questioned whether the treatment actually affected digestibility of the soybean residues.

The prior art methods suffer from disadvantages of requiring high temperature and pressure, partial delignification, and inability of delignification of soybean hull or pod even in sodium chlorite. Thus, there remains a need for a method of converting waste soybean hull fiber into a useful, higher value product with good selectivity, yield and quality.

## SUMMARY OF THE INVENTION

The present invention is a soybean hull or pod fiber which has been delignified and the lignin removed from the remaining cellulose and hemicellulose fibers. Further, the present invention is a method of dissociating the cellulose and hemicellulose as a delignified soybean hull fiber from soybean hull. The soybean hull has a lignin content of no more than 5 weight % and there is no alkaline or hydrogen peroxide treatment involved.

The present invention is a method of delignifying the soybean hull fiber and has the steps of:

(a) exposing the soybean hull to a liquid having water and oxidizer in a non-basic (acidic or neutral) solution thereby separating the lignin from the soybean hull into the liquid; and

(b) separating the cellulose, hemicellulose or combination thereof as a delignified soybean hull fiber from the liquid containing the lignin.

The delignified soybean hull fiber is useful for generating food products, biochemical products, enzyme products and paper products.

The subject matter of the present invention is particularly pointed out and distinctly claimed in the concluding portion of this specification. However, both the organization and method of operation, together with further advantages and objects thereof, may best be understood by reference to the following description taken in connection with accompanying drawings wherein like reference characters refer to like elements.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is spectrum of extracted polyphenolic/lignin component of soyhull.

FIG. 2 is spectrum of filtrate obtained after washing the soybean hull with water.

## DESCRIPTION OF THE PREFERRED EMBODIMENT(S)

The present invention is a delignified soybean hull fiber and method of delignification. The soybean hull initially has cellulose and hemicellulose bound with lignin, wherein the cellulose and hemicellulose are dissociated as a delignified soybean hull fiber. The delignified soybean hull fiber has a lignin concentration less than 5 weight %, preferably less than 1 weight % and most preferably less than or equal to a detection limit of an NMR spectrometer. Soybean hull fiber is yellow/brown looking fiber before delignification. After delignification, it becomes white and fluffy fiber. The lignin or polyphenolic content is removed during delignification and a lignin spectrum is shown in Fig 1.

Conversely, soybean hull fiber is insoluble because the cellulose and hemicellulose is attached or associated by the lignin.

A method of delignifying the soybean hull fiber begins with exposing the soybean hull to a liquid having water and oxidizer in a non-basic solution thereby separating the lignin from the soybean hull into the liquid and separating the cellulose, hemicellulose or combination thereof as a delignified soybean hull fiber from the liquid containing the lignin.

The oxidizer is a strong chemical oxidizer consisting essentially of sodium chlorite. The oxidizer may be provided as an added chemical or may be provided by generating it in solution.

The amount of sodium chlorite may range from greater than zero grams to about 1.25 grams per 5 grams of soybean hull fiber, but is preferably about 0.05 to about 0.25 grams per 5 grams of soybean hull fiber.

The amount of water in the liquid should be at least an amount which saturates the soybean hull fiber, wherein saturation is 100% moisture content.

The amount of water is preferably an amount exceeding 100% saturation, but more preferably an amount exceeding 200% saturation, and most preferably an amount greater than or equal to 300% saturation.

A non-basic solution is a solution with a pH less than 9.0. It is preferred that the solution be about neutral (pH 7.0) or acidic (pH less than 7.0). Preferred pH may be achieved by addition of an acid, preferably a mild acid, for example glacial acetic acid, carbon dioxide or buffer.

It is preferred to provide a sweep gas to remove any chlorinated off gas from the oxidizer.

The delignification reaction may foam. Such foaming may be reduced by addition of an anti-foaming compound, for example octyl alcohol.

The delignification reaction may be carried out at any temperature, but reaction kinetics are improved with increasing temperature. Accordingly, it is preferred to perform the reaction at temperatures above room temperature. At temperatures from about 40 °C to about 75 °C, reaction time is less than 1 hour, generally about 5 minutes. Reactions may be done at higher temperature, but because the mixture is aqueous, pressure containment is needed to preserve a liquid or supercritical phase. Temperatures below 100 °C are preferred to avoid the need for pressurized equipment.

To be useful, the delignified soybean hull fiber is separated from the liquid. The separated delignified soybean hull fiber may further be washed with water to remove any remaining lignin. Removal of water may be done with acetone and drying.

The delignified soybean hull fiber is useful for adding to hemicellulose in papermaking.

The method of the present invention may be extended to add an enzyme, for example cellulase, hemicellulase for example xylanase, arabinase and combinations thereof to the delignified soybean hull fiber for making cellulose and/or hemicellulose derivatives, including but not limited to glucose, xylose, arabinose, polyols, cellulose acetate, cellulose ether and combinations thereof. Cellulose based products include but are not limited to paper.

Example 1

An experiment was conducted to demonstrate delignification of soybean hull fiber. Waste soybean hull fiber from a soybean processing plant was obtained. An amount of 5 g of the soybean hull fiber was mixed with 120 mL of water for a ratio of 24 mL water per g soybean hull fiber. The mixture was heated to 75 °C. An amount of 0.42 mL of glacial acetic acid was added to reduce the pH. An amount of 1.25 g sodium chlorite was gradually added. After 15 minutes the same quantities were added again and repeated for a total of 4 additions having 20 g soybean hull fiber. Nitrogen gas $N_2$ was used as a sweep gas to displace $ClO_2$ reaction gas. Foaming was reduced by addition of 1 –2 drops octyl alcohol. After one hour, the reacted 20 g of soybean hull fiber was rapidly cooled to 20 °C in an ice bath. The products were filtered to separate the delignified soybean hull fiber from the liquid. The delignified soybean hull fiber was washed with water to remove remaining lignin and then further washed with acetone, then dried.

Comparing soybean hull before and after the processing, the color of soybean hull prior to delignification is yellowish or brownish, whereas after delignification, the fiber is whiter due to removal of polyphenolics or lignin component. A filtrate sample extracted during delignification of soybean hull was scanned from 250 nm to 650 nm on a spectrophotometer (Fig 1). The spectrum suggests the presence of polyphenolic molecules, a lignin component. Fig. 2 is the spectrum of wash water showing little or no lignin content because the absorbance is less than 0.06 for all wavelengths.

Example 2

An experiment was conducted to demonstrate conversion of delignified soybean hull fiber to glucose.

A buffered mixture was prepared having a ratio of 5 wt% delignified soybean hull fiber to buffer. The buffer was selected to be compatible with the enzyme. The buffer was sodium acetate in an amount resulting in a pH of 5. The cellulase enzyme was added and permitted to act on the delignified soybean hull

fiber. A control containing no enzyme was identically prepared. Glucose was measured with a glucose enzyme kit.

Results are shown in Table E2-1.

Table E2-1 Delignified Soybean hull Fiber Conversion to Glucose

| Time (Units) | Glucose (mg/ml) |
|---|---|
| 0.0 | 0.05 |
| 1.5 | 0.10 |
| 4.0 | 0.28 |
| 21.5 | 1.02 |

Cellulase enzyme activity assay was performed showing one cellulase unit will produce 1 μmole/hr of glucose from a delignified soybean hull fiber at pH 5.0 and 37 °C. Enzyme activity indicates the presence of glucose as a product. The control with no enzyme produced 0.0 units/mL enzyme activity.

## CLOSURE

While a preferred embodiment of the present invention has been shown and described, it will be apparent to those skilled in the art that many changes and modifications may be made without departing from the invention in its broader aspects. The appended claims are therefore intended to cover all such changes and modifications as fall within the true spirit and scope of the invention.

# CLAIMS

We claim:

1. A delignified soybean hull fiber comprising dissociated cellulose and hemicellulose and an amount of polyphenolics or lignin less than 5 weight %.

2. The soybean hull fiber as recited in claim 1, wherein the amount of polyphenolics or lignin is less than 1 weight %.

3. The soybean hull fiber as recited in claim 1, wherein the amount of polyphenolics or lignin is less than or equal to a detection limit of an NMR spectrometer.

4. The soybean hull fiber as recited in claim 1, wherein said delignified soybean hull fiber is soluble in a carbohydrate dissolving solvent.

5. The soybean hull fiber as recited in claim 1, wherein said delignified soybean hull fiber is further acid hydrolyzed to produce C5 and C6 sugars.

6. The soybean hull fiber as recited in claim 1, wherein said delignified soybean hull fiber is further converted to obtain cellulose based products, food additives, and food products.

7. A method of delignifying a soybean hull having cellulose, hemicellulose and lignin, comprising the steps of:

(a) exposing the soybean hull to a liquid having water and an oxidizer in a non-basic solution thereby separating the lignin from the soybean hull into the liquid; and

(b) separating the cellulose, hemicellulose or combination thereof as a delignified soybean hull fiber from the liquid containing the lignin.

8. The method as recited in claim 7, wherein said separating includes washing the delignified soybean hull fiber with water to remove the remaining lignins.

9. The method as recited in claim 7, wherein said oxidizer is sodium chlorite.

10. The method as recited in claim 9, wherein said sodium chlorite is in an amount ranging from greater than zero grams of said sodium chlorite per about 5 grams of soybean hull to about 1.25 grams of said sodium chlorite per about 5 grams of soybean hull.

11. The method as recited in claim 7, wherein said water is in an amount at least about 200% of the saturation point of said soybean hull.

12. The method as recited in claim 7, wherein said liquid has a pH less than 9.

13. The method as recited in claim 12, wherein the pH is about acidic.

14. The method as recited in claim 12, wherein the pH is about neutral.

15. The method as recited in claim 7, wherein the temperature of said exposing the soybean hull to a liquid oxidizer is from about 40 °C to about 75 °C.

16. The method as recited in claim 7, further comprising the steps of:

(a) preparing a mixture of said cellulose, hemicellulose or a combination thereof with a buffer; and

(b) adding a cellulase enzyme and obtaining glucose.

17. The method as recited in claim 7, further comprising the step of adding the cellulose, hemicellulose or a combination thereof to a fiber stream in a paper mill.

18. The method as recited in claim 7, wherein said oxidizer is obtained by generating the oxidizer in an electrochemical process.

19. The method as recited in claim 18, wherein said electrochemical process is selected from the group consisting of gas plasma, corona discharge and combinations thereof.

20. A method of delignifying a soybean hull having cellulose, hemicellulose and lignin, comprising the steps of:

(a) exposing the soybean hull to a liquid having water and an oxidizer in a non-basic solution thereby separating the lignin from the soybean hull into the liquid; and

(b) separating the cellulose, hemicellulose or a combination thereof as a delignified soybean hull fiber from the liquid containing the lignin.

(c) washing the delignified soybean hull fiber with water to remove the remaining lignins.

21. The method as recited in claim 20, wherein said oxidizer is sodium chlorite.

22. The method as recited in claim 21, wherein said sodium chlorite is in an amount ranging from greater than zero grams of said sodium chlorite per about 5 grams of soybean hull to about 1.25 grams of said sodium chlorite per about 5 grams of soybean hull.

23. The method as recited in claim 20, wherein said water is in an amount at least about 200% of the saturation point of said soybean hull.

24. The method as recited in claim 20, wherein said liquid has a pH less than 9.

25. The method as recited in claim 24, wherein the pH is about acidic.

26. The method as recited in claim 24, wherein the pH is about neutral.

27. The method as recited in claim 20, wherein the temperature of said exposing the soybean hull to a liquid oxidizer is from about 40 °C to about 75 °C.

28. The method as recited in claim 20, further comprising the steps of:

(a) preparing a mixture of said cellulose, hemicellulose or a combination thereof with a buffer; and

(b) adding a cellulase enzyme and obtaining glucose.

29. The method as recited in claim 20, further comprising the step of adding the cellulose, hemicellulose or a combination thereof to a fiber stream in a paper mill.

30. The method as recited in claim 20, wherein said oxidizer is obtained by generating the oxidizer in an electrochemical process.

31. The method as recited in claim 30, wherein said electrochemical process is selected from the group consisting of gas plasma, corona discharge and combinations thereof.

32. A method of delignifying a soybean hull having cellulose, hemicellulose and lignin, comprising the steps of:

(a) exposing the soybean hull to a liquid having water and an oxidizer in a non-basic solution thereby separating the lignin from the soybean hull into the liquid, said water in an amount at least about 200% of the saturation point of said soybean hull; and

(b) separating the cellulose, hemicellulose or a combination thereof from the liquid.

33. The method as recited in claim 32, wherein said separating includes washing the delignified soybean hull fiber with water to remove the remaining lignins.

34. The method as recited in claim 32, wherein said oxidizer is sodium chlorite.

35. The method as recited in claim 34, wherein said sodium chlorite is in an amount ranging from greater than zero grams of said sodium chlorite per about 5 grams of soybean hull to about 1.25 grams of said sodium chlorite per about 5 grams of soybean hull.

36. The method as recited in claim 32, wherein said water is in an amount at least about 200% of the saturation point of said soybean hull.

37. The method as recited in claim 32, wherein said liquid has a pH less than 9.

38. The method as recited in claim 37, wherein the pH is about acidic.

39. The method as recited in claim 37, wherein the pH is about neutral.

40. The method as recited in claim 32, wherein the temperature of said exposing the soybean hull to a liquid oxidizer is from about 40 °C to about 75 °C.

41. The method as recited in claim 32, further comprising the steps of:

(a) preparing a mixture of said cellulose, hemicellulose or a combination thereof with a buffer; and

(b) adding a cellulase enzyme and obtaining glucose.

42. The method as recited in claim 32, further comprising the step of adding the cellulose, hemicellulose or a combination thereof to a fiber stream in a paper mill.

43. The method as recited in claim 32, wherein said oxidizer is obtained by generating the oxidizer in an electrochemical process.

44. The method as recited in claim 43, wherein said electrochemical process is selected from the group consisting of gas plasma, corona discharge and combinations thereof.

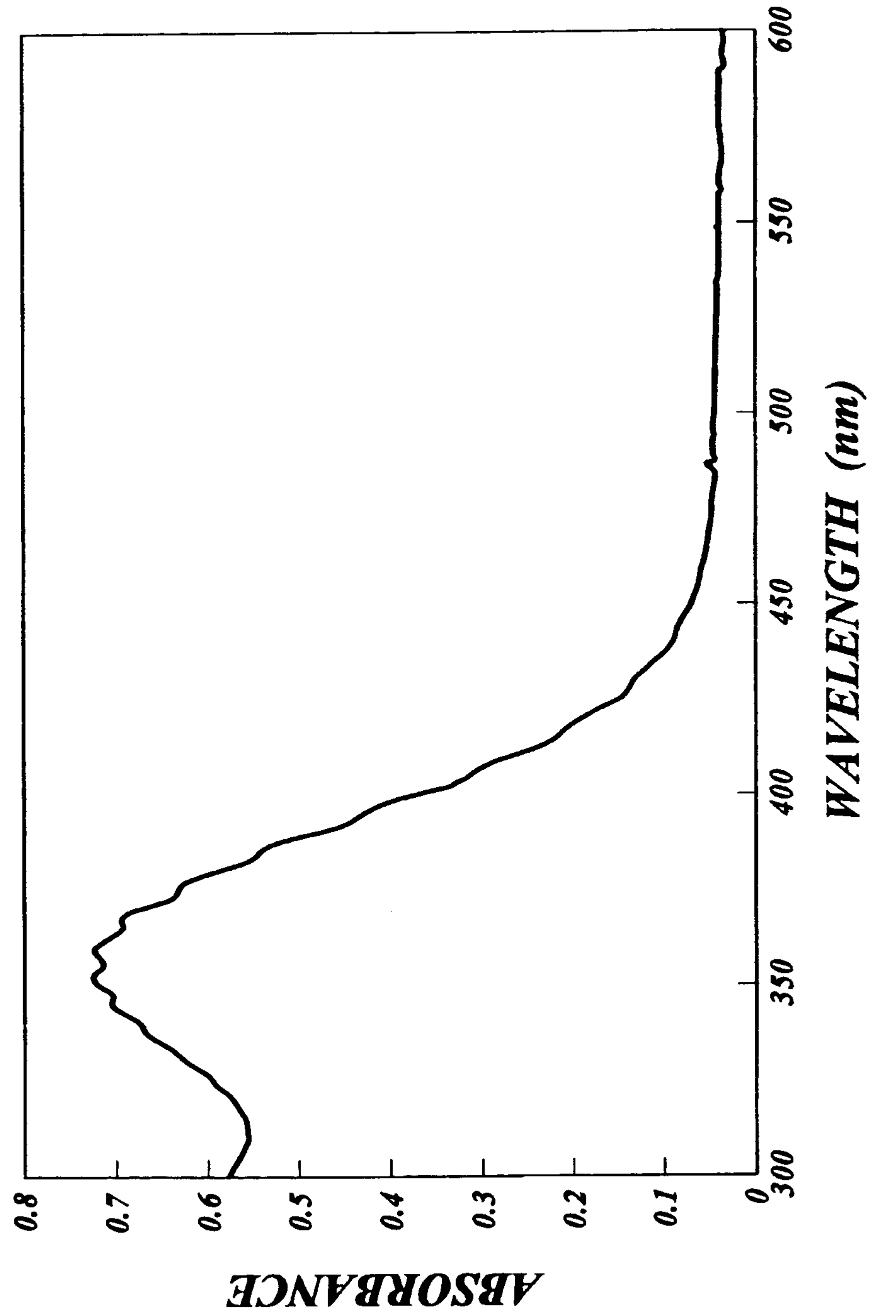
ABSORBANCE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
WAVELENGTH (nm)
300
350
400
450
500
550
600

*Fig. 1*

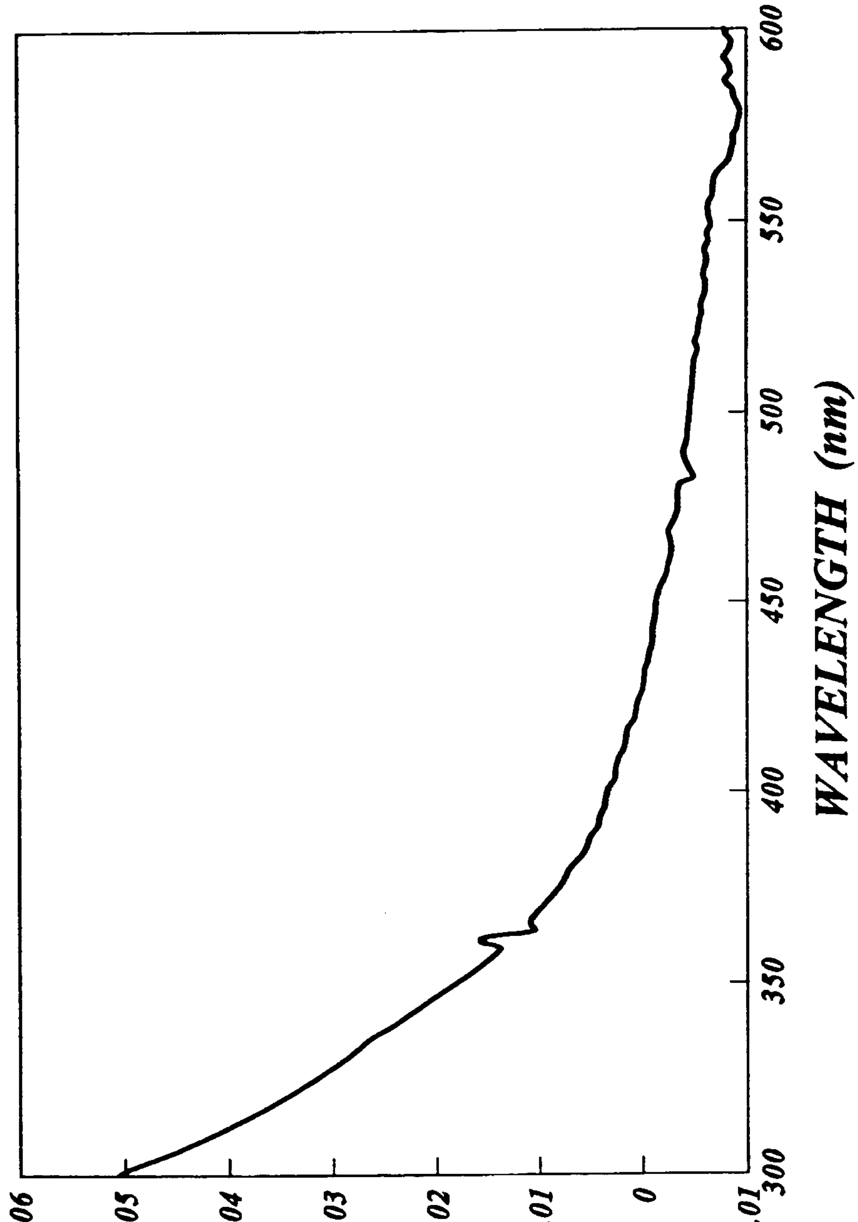
ABSORBANCE
0.06
0.05
0.04
0.03
0.02
0.01
0
-0.01
300
350
400
450
500
550
600
WAVELENGTH (nm)

*Fig. 2*

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/23380

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 D21C5/00

According to International Patent Classification (IPC) or to both national classification and IPC

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IPC 7 D21C

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5 705 216 A (TYSON GEORGE J) 12 August 1998 (1998-08-12) cited in the application column 12, line 20 - line 44 claim 2 | 1-6,8,11 |
| Y | the whole document | 7,9,10, 12-44 |
| X | US 4 842 877 A (TYSON GEORGE J) 27 June 1989 (1989-06-27) & US 5 023 097 A (TYSON GEORGE J) 27 June 1989 (1989-06-27) figure column 5, line 40 - line 51 column 11, line 33 - line 42 | 1-6,8,11 |
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | DATABASE WPI<br>Section Ch, Week 198512<br>Derwent Publications Ltd., London, GB;<br>Class D13, AN 1985-072246<br>XP002129426<br>& JP 60 027365 A (NIPPON SHOKUHIN KAKO KK)<br>, 12 February 1985 (1985-02-12)<br>abstract | 16,28,41 |
| A | US 5 656 129 A (GOOD DAVID BENARD ET AL)<br>12 August 1997 (1997-08-12)<br>cited in the application | 1-44 |
| A | In Vitro And In Vivo Evaluations Of<br>Soybean Residues Ensiled With Various<br>Additives, J. animal sci. 49, no.6:<br>1545-1551 (1979), Miller et al<br>XP002129425<br>cited in the application | 9,21,34 |
| A | US 4 997 665 A (GRETHLEIN HANS)<br>5 March 1991 (1991-03-05)<br>abstract; examples | 1-44 |
| A | US 4 472 254 A (DOTSON RONALD L ET AL)<br>18 September 1984 (1984-09-18)<br><br>the whole document | 18,19,<br>30,31,<br>43,44 |
| A | PATENT ABSTRACTS OF JAPAN<br>vol. 1996, no. 01,<br>31 January 1996 (1996-01-31)<br>& JP 07 238488 A (BIO POLYMER RES:KK),<br>12 September 1995 (1995-09-12)<br>abstract | 17,29,42 |

2

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/23380

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 5705216 A | 06-01-1998 | NONE | |
| US 4842877 A | 27-06-1989 | AT 98438 T | 15-01-1994 |
| | | AU 3535989 A | 03-11-1989 |
| | | CA 1329046 A | 03-05-1994 |
| | | DE 68911525 D | 27-01-1994 |
| | | DE 68911525 T | 21-07-1994 |
| | | EP 0415959 A | 13-03-1991 |
| | | WO 8909547 A | 19-10-1989 |
| | | US 5023097 A | 11-06-1991 |
| JP 60027365 A | 12-02-1985 | JP 1755689 C | 23-04-1993 |
| | | JP 4045146 B | 23-07-1992 |
| US 5656129 A | 12-08-1997 | CN 1141374 A | 29-01-1997 |
| | | JP 9225908 A | 02-09-1997 |
| US 4997665 A | 05-03-1991 | CA 2026696 A | 06-04-1991 |
| | | EP 0421758 A | 10-04-1991 |
| | | JP 3180156 A | 06-08-1991 |
| US 4472254 A | 18-09-1984 | NONE | |
| JP 07238488 A | 12-09-1995 | JP 2798882 B | 17-09-1998 |

# HPS Trailer Page
for
# EAST

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**Printer:** cp3_6c23_gbgpptr

## Summary

| Document | Pages | Printed | Missed | Copies |
|---|---|---|---|---|
| WO000020677 | 21 | 21 | 0 | 1 |
| Total (1) | 21 | 21 | 0 | - |